arXiv:cond-mat/0401299v1 [cond-mat.soft] 16 Jan 2004

Effect of Bilayer Thickness on Membrane Bending Rigidity

H. Bermúdez, D. A. Hammer, and D. E. Discher

Department of Chemical and Biomolecular Engineering,

University of Pennsylvania, Philadelphia, Pennsylvania 19104

Abstract

The bending rigidity k_c of bilayer vesicles self-assembled from amphiphilic diblock copolymers has been measured using single and dual-micropipet techniques. These copolymers are nearly a factor of 5 greater in hydrophobic membrane thickness d than their lipid counterparts, and an order of magnitude larger in molecular weight $\bar{M}_n$. The macromolecular structure of these amphiphiles lends insight into and extends relationships for traditional surfactant behavior. We find the scaling of k_c with thickness to be nearly quadratic, in agreement with existing theories for bilayer membranes. The results here are key to understanding and designing soft interfaces such as biomembrane mimetics.

I. INTRODUCTION

Thin films of surfactants are found in numerous contexts, ranging from emulsions and colloids to biological membranes.[1] The bending rigidity of such films is a key determinant of many structures and processes including cell shape, fusion, and adhesion. Surfactant studies to date have been largely limited in scope by synthetic chemistry capabilities, and thus, generalizations of emergent properties have been similarly constrained. Several groups have nevertheless developed theoretical and numerical methods to predict properties of monolayers and bilayers from both continuum and molecular perspectives.[2,3,4,5,6] Experimentally, the advent of techniques such as living anionic polymerization[7] has provided the means to create relatively monodisperse amphilphilic diblock copolymers – the macromolecular analogues to "short" chain surfactants. Besides finding novel uses in the above contexts,[8] these macromolecules also serve to test the limits of existing theories.

In this letter, we examine the dependence of the bending rigidity k_c on the hydrophobic thickness d of closed bilayer membranes (vesicles). Other important properties that vary with d, such as permeability and elasticity, are discussed elsewhere.[9,10] From the simplest models, a bilayer can be pictured as being composed of thin elastic shell(s). The deformation of such a shell[2,11] is given by the 2-D Lame coefficients μ and λ and the shell thickness l. It follows that the area elastic modulus $K_A = \lambda + \mu$ and the bending rigidity $k_c = (\lambda + 2\mu)l^2/12$. Taking the shear modulus $\mu = 0$ for fluid membranes leads to the often-cited result,[5,6]

$$k_c = \beta K_A d^2 \tag{1}$$

(where β is a constant). This relation serves as motivation for our study. Any possible interdigitation in the bilayer does not affect the scaling of k_c, but is instead reflected in the prefactor β.[2,11] When the membrane is taken as uncoupled monolayers free to slide past one another, $\beta = 1/48$, and when taken as completely coupled monolayers, $\beta = 1/12$. More complex descriptions[3] include effects such as chain flexibility and associated entropy, with $k_c \sim d^{2.5}$. Note that for a rigid plate,[12] $k_c \sim d^3$, and thus we expect a strong scaling dependence, regardless of the exact details.

The picture of a biological membrane is clearly more complicated, as it is perforated by integral proteins and has associated proteins attached to its surfaces. In particular, the presence of a surface brushy layer, or glycocalyx, is likely to contribute to bending resistance,

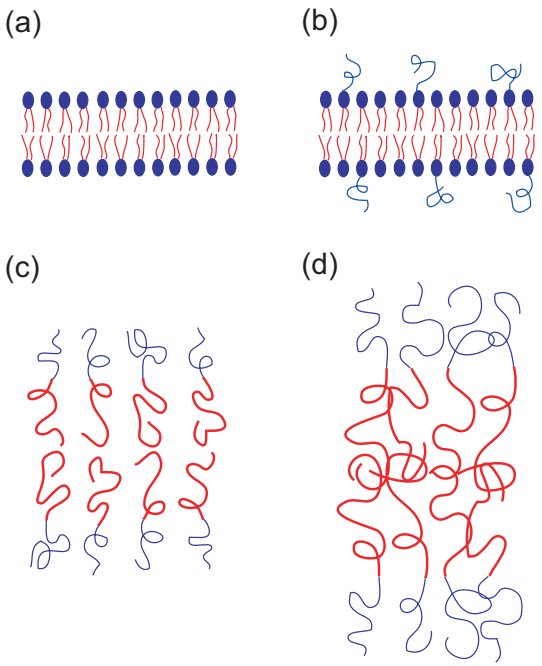

Figure 1: Schematic of different bilayer membrane configurations. (a) Typical phospholipid membrane of hydrophobic thickness $d \approx 3$ nm. (b) PEO-conjugated lipids can typically be accommodated only up to 15 mol % before micellization begins. At this low grafting density, PEO is in the mushroom or marginal brush regime.[17] (c) Diblock copolymer membranes are much thicker ($d > 8$ nm), and the PEO is expected to be near or in the brush regime.[18] (d) At still larger d, chains may become entangled either laterally within a monolayer or across monolayers.

as well as other properties and processes.[13] Brushes on lipid membranes have been studied to a limited extent by attaching poly(ethylene oxide) (PEO) to phospholipid headgroups. These "Stealth" vesicles have found wide use in drug delivery applications,[14] and they presumably suppress immune response by means of the surface steric stabilization imparted by PEO. However, lipid vesicles generally cannot accommodate more than $\approx$ 15 mol % of PEO-conjugated lipid due to resulting curvature effects and subsequent micellization.[15,16] This limitation on the amount of PEO that can be incoporated into lipid membranes implies that the PEO is probably in a mushroom or marginal brush configuration at best.[17]

In contrast to lipid membranes (and perhaps closer to biomembranes), polymer membranes have a dense PEO layer, likely in a brush or partially collapsed brush state.[18] The effect of brushes on membrane elasticity and rigidity has been studied theoretically by sev-

eral groups,[19,20] but experimental verifications with lipid-based systems[21] are limited for the reasons mentioned above. Various model scenarios are depicted schematically in Figure 1. While it may not be possible to experimentally decouple the contributions of the hydrophobic and hydrophilic segments, direct measurements over a broad range of d can still yield insight into general membrane behavior.

Table I: Structural details of vesicle-forming amphiphiles. The common biomembrane lipids SOPC (1-stearoyl-2-oleoyl phosphatidylcholine) and DMPC (1,2-dimyristoyl phosphatidylcholine) are included for comparison. The hydrophilic fraction $f \approx 0.3-0.4$ is consistent with lamellar structures. Cryo-TEM provides direct measures of the hydrophobic thickness d.[10]

Amphiphile	Polymer Formula	$\bar{M}_n$ (kg/mol)	f	d (nm)
DMPC	—	0.68	≈ 0.36	2.5
SOPC	—	0.79	≈ 0.31	3.0
OE7	PEO_{40}-PEE_{37}	3.9	0.39	8.0
OB2	PEO_{26}-PBD_{46}	3.6	0.28	9.6
OB18	PEO_{80}-PBD_{125}	10.4	0.29	14.8

II. EXPERIMENTAL METHODS

The polymers of PEO-polybutadiene (PEO-PBD) as well as PEO-poly(ethylethylene) (PEO-PEE) were synthesized by standard living anionic polymerization techniques.[22] The number of monomer units in each block was determined by ^{1}H NMR. Gel permeation chromatography with polystyrene standards was used to determine number-average molecular weights $\bar{M}_n$ as well as polydispersity indices (always < 1.10) (Table I). The hydrophobic membrane thickness d was previously determined by direct imaging of vitrified samples with cryogenic transmission electron microscopy (cryo-TEM).[10] Measurements of related micelles via cryo-TEM agree well with independent small-angle neutron scattering (SANS) results.[18,23]

Giant vesicles were prepared by typical film rehydration techniques[24] and imaged under bright-field optics to provide distinct imaging of the vesicle membrane. Narishige manipu-

lators were connected to a custom manometer system with pressure transducers (Validyne, Northridge, CA) for control and monitoring of the aspiration pressure. To obtain small pressures (≈ 10 Pa), Mitutoyo digital micrometers were used to displace the relative heights of water in the manometer system. In the micromanipulation technique,[25,26] a giant vesicle is made slightly flaccid and then aspirated into a micropipet. From vesicle geometry, the applied pressure, and the aspirated projection length, one can calculate the imposed membrane tension Σ and the relative area dilation $\alpha \equiv \Delta A/A_o$. The area dilation is related to the membrane elastic constants, and is generally written[27,28] as a superposition of the entropic elasticity of surface undulations and direct membrane stretching against cohesive forces:

$$\alpha = (k_B T/8\pi k_c)\ln(1 + cA\Sigma) + \Sigma/K_A, \tag{2}$$

where the coefficient c (≈ 0.1) depends on the type of mode expansion used to describe the undulations (*e.g.,* plane wave or quasispherical approach). From Eq. (2), k_c is directly obtained from a plot of $\ln(\Sigma)$ versus α.

Here we use two different, but related, micropipet aspiration techniques. Soft membranes, those with k_c about $10 - 100k_BT$, will exhibit commensurate surface undulations. These fluctuations can be analyzed optically[29] or be suppressed with a micropipet[30] (Fig. 2a). We have chosen the latter approach, since it also gives other material properties such as interfacial elasticity in a single measurement. For stiffer membranes ($k_c > 100k_BT$) thermal fluctuations are suboptical and potentially dampened by viscous dissipation within the bilayer, requiring other methods to determine k_c. One particular micropipet approach was developed by Zhelev and co-workers to study neutrophils,[31] which are quite stiff because of their highly viscous cytoplasmic interiors. Initial observations of polymer vesicles formed from **OB18** (PEO_{80}-PBD_{125}) and higher $\bar{M}_n$ polymers indicated that viscous effects were not negligible,[10] and thus would be better suited for this dual-pipet technique. By simultaneously aspirating a vesicle with two pipets (Fig. 3a), and accounting for the energy of the deformed regions, one obtains:

$$\Delta P_s = f_0 k_c/R_{ps}^3 + f_1 \Delta P_l/R_{ps} \tag{3}$$

where the coefficients f_0 and f_1 are functions of the pipet and vesicle geometry.[31] Aspiration pressures and pipet radii are denoted by ΔP and R_p, respectively, and the subscripts

l and s distinguish between large and small pipets. From Eq. (3), k_c is obtained from the intercept of ΔP_s vs. ΔP_l.

III. RESULTS AND DISCUSSION

The use of different techniques is necessitated by the strong effect of d on membrane properties. Although the present study of diblocks is still limited in size, it considerably extends the range of membrane thickness over prior studies[26] with lipid vesicles ($d = 2.4-3.4$ nm). A further difficulty with lipid or "short" chain systems is the ambiguity regarding the applicability of polymer theory. Polymeric systems are able to mitigate both of these limitations by virtue of their macromolecular nature.

Observations of vesicles made from **OE7** (PEO_{40}-PEE_{37}) and **OB2** (PEO_{26}-PBD_{46}) indicated that these membranes were relatively lipid-like and hence would have fairly low values of k_c. It is important to note that microscopic fluctuations persist at all levels of tension, thereby renormalizing the area elastic modulus.[30] By virtue of their increased thickness, polymer membranes have larger k_c and maximum areal strains α_c,[10] and thus the correction to K_A is less than 5%. For thinner and softer membranes, the apparent elastic modulus $K_{app} \equiv \partial\Sigma/(\partial A/A)$ is related to the true modulus K_A by

$$K_A/K_{app} = 1 + K_A k_B T/8\pi k_c \Sigma. \tag{4}$$

From Eq. (4), the crossover tension Σ_x between the regime dominated by fluctuations and the regime dominated by direct expansion is expected to decrease with d, since $\Sigma_x = K_A k_B T/8\pi k_c \sim d^{-2}$. Indeed we find for **OB2** a lower value of Σ_x compared to lipids with $k_c = 24.7 \pm 11.1\ k_B T$ ($N = 6$). As a control, we also examined the prototypical lipid SOPC (1-stearoyl-2-oleoyl phosphatidylcholine) having $d = 3$ nm, and found $k_c = 16.4 \pm 6.9\ k_B T$ ($N = 9$), in good agreement with published values.[26] For the thickest membrane studied here, **OB18**, measurements with the dual pipet technique give $k_c = 466 \pm 157\ k_B T$ ($N = 4$), substantially larger than any lipid (Table II and Fig. 3).

At first glance it may seem surprising that **OB2** has a lower value of k_c than **OE7**, even though it has a thicker hydrophobic core d. However, the PEO contribution to the overall membrane thickness might also need to be considered.[21,32] The contrast from PEO via cryo-TEM is rather limited, essentially prohibiting direct measurement of the corona by

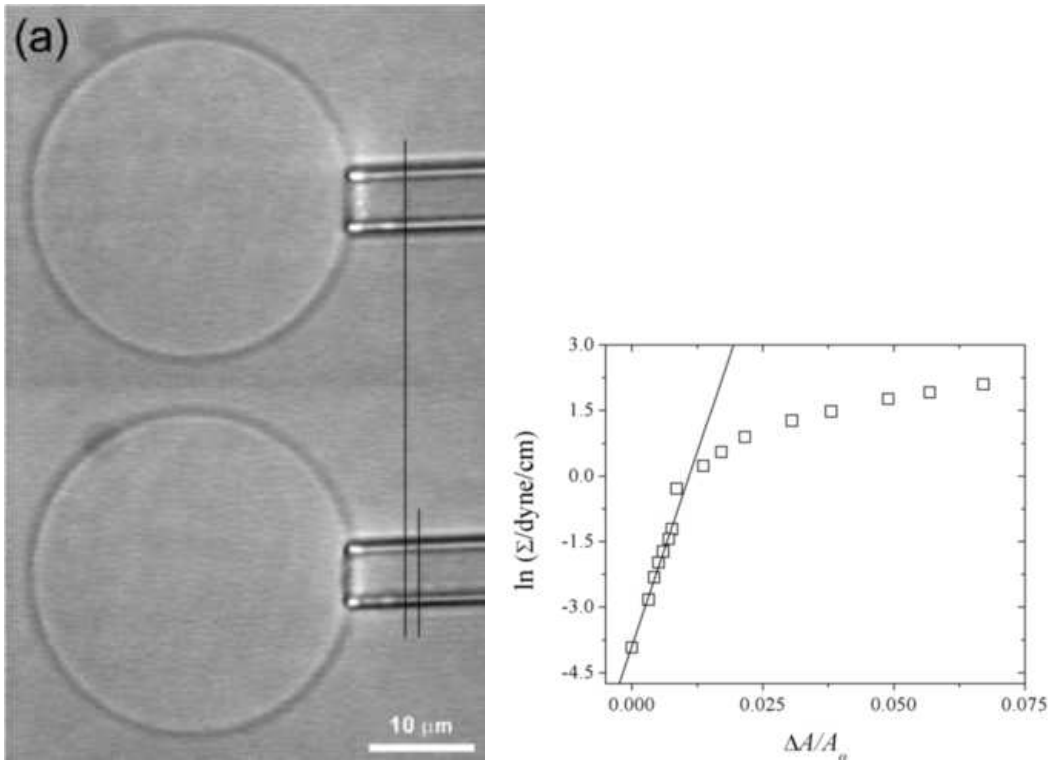

Figure 2: Single pipet measurements of the bending rigidity k_c (*e.g.*, **OB2**). (a) Micropipet aspiration suppresses the membrane fluctuations at low tensions, smoothing out excess area in the membrane and resulting in an increased projection length ΔL.[30] (b) Corresponding plot of imposed tension vs. area dilation to determine k_c. The slope is $8\pi k_c/k_BT$ from Eq. (2).

this technique. We can however, make an estimate of this contribution from polymer theory. Inspection of Table I reveals that **OB2** has a shorter PEO chain than **OE7**. Evidence for a brush or partially collapsed PEO brush in related diblock micelles[18] suggests that the PEO chains are stretched relative to a Gaussian state. Given the large incompatibility between PEO and PBD, a conservative yet reasonable estimate of the PEO length would come from assuming these diblocks are in the strong segregation limit (SSL). In the SSL, the characteristic domain length scales with molecular weight as $R \sim N^{2/3}$, with the result that **OE7** should be about 10% thicker than **OB2**. While this difference is modest, it may be sufficient to explain the data given the strong dependence of k_c with membrane thickness.

Single pipet measurements were not performed with **OB18** but it is expected that any such attempts would be frustrated by long response times and viscous dissipation within the membrane. For a $d = 15$ nm membrane, Eq. (4) predicts $\Sigma_x \approx 1 \times 10^{-2}$ dyne/cm, indicating

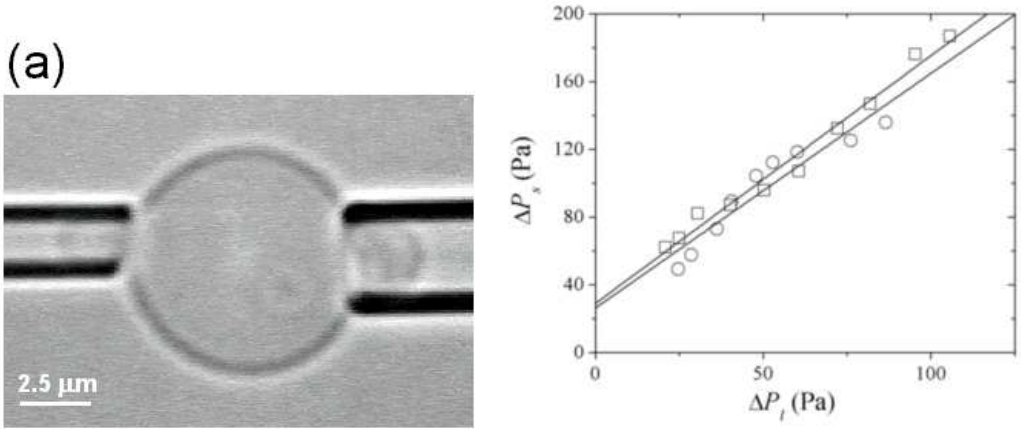

Figure 3: Dual pipet measurement of the bending rigidity k_c (*e.g.*, **OB18**). (a) A vesicle is partially aspirated into two pipets with different radii (R_{pl} and R_{ps}). The larger pipet suction ΔP_l is used to take up excess area, while the smaller pipet aspiration pressure ΔP_s, deforms a small region of the membrane. (b) Corresponding aspiration curves for two different vesicles. The thermodynamic analysis of Zhelev *et al.*,[31] leading to Eq. (3), is used to extract k_c.

Table II: Material properties of polymer and lipid vesicles. The "coupling" constant β as obtained from Eq. (1). $*$ Literature values for selected systems.[9,26]

Amphiphile	K_A (N/m)	k_c (k_BT)	β
DMPC*	0.234	13.3 ± 1.4	1/26
SOPC*	0.235	21.4 ± 1.4	1/24
SOPC	0.203	16.4 ± 6.9	1/27
OE7*	0.120	33.3 ± 7.1	1/55
OB2	0.098	24.7 ± 11.1	1/90
OB18	0.109	465.5 ± 157	1/13

the difficulty in measuring k_c by this technique. Thicker membranes ($d > 15$ nm) are predisposed to viscous and entanglement effects,[10] and were therefore not pursued further. Similarly, we did not carry out dual pipet measurements for our thinner membranes. As has been previously noted by Zhelev *et al.*,[31] dual pipet measurements are not very sensitive

and hence are best suited for much stiffer membranes where such errors can be tolerated.

Combining the above results in Figure 4 shows good agreement with mean-field predictions for k_c, even though these systems are quite different from the classic phospholipid and surfactant systems motivating these theories. Rescaling these chemically distinct amphiphiles by the elastic area modulus K_A is essential: for fluid membranes (such as these[33]), it has been shown both theoretically[34] and experimentally[10] that K_A is controlled by details of the interfacial chemistry and *not* by the thickness d. The extent of interdigitation is described by the numerical prefactor β, which in principle could vary with d, amphiphile persistence length, or chemistry. Thus it is not so surprising that SOPC and **OB2** have low values of β, given their visibly soft nature (Table II). For **OB18**, Eq. (1) gives $\beta = 1/13$, implying that its leaflets are essentially coupled together. This result is consistent with other observations that **OB18** is a highly viscous, and possibly entangled, membrane.[33] Rigorously, both β *and* K_A are needed to determine the scaling, but we do not have independent measurements of the former. It is interesting to note that the product βK_A may remain constant by having leaflet coupling (structure) offset changes in the interfacial tension (chemistry).

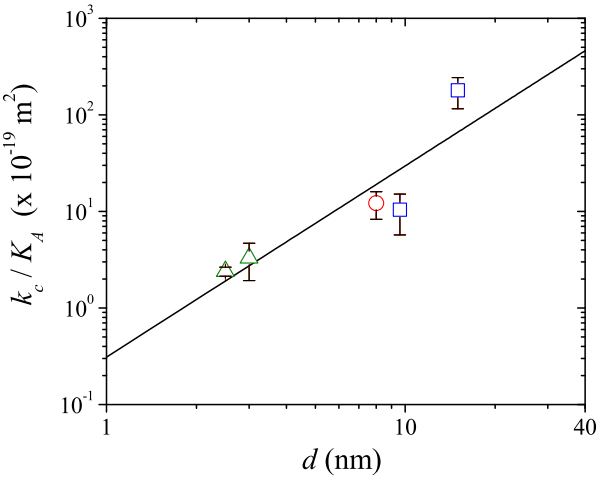

Figure 4: Scaling of k_c with membrane hydrophobic thickness d. Data are shown for DMPC (△),[26] SOPC (△), **OE7** (○),[9] **OB2** (□) and **OB18** (□) vesicles. **OB18** data are obtained by dual-pipet measurements, whereas all other data come from single-pipet measurements. Line is a least-squares fit, giving a scaling exponent of 2.0 ($R^2 = 0.809$) as predicted by Eq. (1).

IV. CONCLUSIONS

Sensitive micropipet techniques are used to examine single vesicles and extract the bending rigidity k_c for membranes self-assembled from amphiphilic diblock copolymers. The macromolecular nature of these amphiphiles considerably broadens the range over which these systems can be studied, even to the point where bulk effects arise. We find the scaling of k_c with thickness d to be nearly quadratic, in agreement with existing theories for bilayer membranes. The results will likely influence future work on extending surfactant assemblies, since bending is often a predominant mode of deformation in soft matter systems. Future work with mixing of short and long chains (*e.g.*, **OB2** with **OB18**) is expected to cause a dramatic lowering of k_c and is yet another means of controlling interfacial properties.

Acknowledgments

The authors thank the Bates group at the University of Minnesota for synthesis and characterization of the copolymers in Table I. Funding was provided by NSF-MRSEC and NASA.

---

(1) Safran, S.A. *Statistical Thermodynamics of Surfaces, Interfaces, and Membranes*; Westview: New York, 2003.

(2) Bloom, M; Evans, E.; Mouritsen, O.G. *Q. Rev. Biophys.* **1991**, *24*, 293.

(3) Szleifer, I.; Kramer, D.; Ben-Shaul, A.; Roux, D.; Gelbart, W.M. *Phys. Rev. Lett.* **1988**, *60*, 1966.

(4) Goetz, R.; Gompper, G.; Lipowsky, R. *Phys. Rev. Lett.* **1999**, *82*, 221.

(5) Evans, E. *Biophys. J.* **1974**, *14*, 923.

(6) Helfrich, W. *Z. Naturforsch.* **1975**, *30c*, 841.

(7) Webster, O. *Science* **1991**, *251*, 887.

(8) Alexandridis, P. and Lindmann, B. In *Amphiphilic Block Copolymers: Self-Assembly and Applications*; Elsevier: New York, 2000.

(9) Discher, B.M.; Won, Y.-Y.; Ege, D.S.; Lee, J.C.-M.; Bates, F.S.; Discher, D.E.; Hammer, D.A *Science* **1999**, *284*, 1143.

(10) Bermudez, H.; Brannan, A.K.; Hammer, D.A.; Bates, F.S.; Discher, D.E. *Macromolecules* **2002**, *35*, 8203.

(11) Landau, L. and Lifshitz, L. *Theory of Elasticity*; Oxford: New York, 1970.

(12) Boal, D. *Mechanics of the Cell*; Cambridge: New York, 2002.

(13) Lieber, M.R. and Steck, T.L. *J. Biol. Chem.* **1982**, *257*, 11660.

(14) Lasic, D.D. and Papahadjopoulos, D. *Medical applications of liposomes*; Elsevier: New York, 1998.

(15) Belsito, S.; Bartucci, R.; Montesano, G.; Marsh, D.; Sportelli, L. *Biophys. J.* **2000**, *78*, 1420; Montesano, G.; Bartucci, R.; Belsito, S.; Marsh, D.; Sportelli, L. *Biophys. J.* **2001**, *80*, 1372.

(16) Israelachvili, J.N. *Surface and Intermolecular Forces*; Academic Press: New York, 1992.

(17) de Gennes, P.-G. *Scaling Concepts in Polymer Physics*; Cornell University Press: Ithaca, NY, 1979.

(18) Won, Y.-Y.; Davis, H.T.; Bates, F.S.; Agamalian, M.; Wignall, G.D. *J. Phys. Chem. B* **2000**, *104*, 7134.

(19) Laradji, M. *Europhys. Lett.* **2002**, *60*, 594.

(20) Bickel, T. and Marques, C.M. *Eur. Phys. J. E* **2002**, *9*, 349.

(21) Evans, E. and Rawicz, W. *Phys. Rev. Lett.* **1997**, *79*, 2379.

(22) Hillmyer, M.A. and Bates, F.S. *Macromolecules* **1996**, *29*, 6994.

(23) Won, Y.-Y., Davis, H.T., Bates, F.S. *Science* **1999**, *283*, 960.

(24) Lee, J.C-M., Bermudez, H, Discher, B.M., Sheehan, M.A., Won, Y.-Y., Bates, F.S., Discher, D.E. *Biotechnol. Bioeng.* **2001**, *73*, 135.

(25) Evans, E.A and Skalak, R. *Mechanics and Thermodynamics of Biomembranes*; CRC Press: Boca Raton, FL, 1980.

(26) Rawicz, W.; Olbrich, K.C.; McIntosh, T; Needham, D.; Evans, E. *Biophys. J.* **2000**, *79*, 328.

(27) Helfrich, W. and Servuss, R.-M. *Nuovo Cimento* **1984**, *D3*, 137.

(28) Milner, S. and Safran, S.A. *Phys. Rev. A* **1987**, *36*, 4371.

(29) Döbereiner, H.-G.; Evans, E.; Kraus, M.; Seifert, U.; Wortis, M. *Phys. Rev. E* **1997**, *55*, 4458

(30) Evans, E. and Rawicz, W. *Phys. Rev. Lett.* **1990**, *64*, 2094.

(31) Zhelev, D.V.; Needham, D; Hochmuth, R.M. *Biophys. J.* **1994**, *67*, 720.

(32) Castro-Roman, F.; Porte, G.; Ligoure, C. *Phys. Rev. Lett.*, **1999**, *82*, 109.

(33) Lee, J.C.-M.; Santore, M.; Bates, F.S.; Discher, D.E. *Macromolecules* **2002**, *35*, 323.

(34) Helfand, E. and Wasserman, Z.R. In *Developments in Block Copolymers*; Ed. Goodman, I.; Applied Science: New York, 1982.